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DEVELOPMENT IN *PODOCARPUS NIVALIS* IN RELATION TO OTHER PODOCARPS.

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In the first paper of this series (Boyle and Doyle, 1953) development of the gametophytes, the male from pollination and the female from the first appearance of the rudiment of the ovule, was followed in *P. nivalis* up to fertilization, thus giving a reasonably full account of these stages in a typical member of the *Eupodocarpus* section of *Podocarpus*. Comparisons were made with corresponding stages in the other sections of *Podocarpus* and in the other genera, enabling tentative suggestions to be made concerning the nature of the basal gametophytes and of gametophytic developmental tendencies in the Family. In the second paper (Boyle and Doyle, 1954) the embryogeny of *P. nivalis* was followed from fertilization up to the organization of the multicellular clumps which are the potential embryos. In this second paper comparisons were made with the embryogenies shown by other members of the *Eupodocarpus* section, and it was shown that the sequence of development in *P. nivalis* is, within certain limits, typical of the section.

In the present paper comparisons are extended and this embryogeny including cleavage polyembryony, typical of the *Eupodocarpus* section, is set against the available data for embryogeny in the rest of the Podocarpaceae with a view to determine, if possible, the nature of the basal embryogeny in the Family and the derivative tendencies in embryological development shown within it. In summary there is put forward a preliminary picture in general terms of the nature of the whole development in one family of conifers.

In this discussion a few points may be introduced which have been briefly touched upon in earlier papers (*cf.* Doyle and Looby, 1939; Looby and Doyle, 1944b). Where this is done it is to give continuity to the considerations involved, or because such points can be more adequately treated now in the light of increasing knowledge.

Embryological Comparisons with Other Podocarps.

(*A*) *General.*

The *plan* of proembryo development followed out in *P. nivalis* seems common to all podocarps though details may vary considerably. The plan is the succession of (*a*) a free nuclear stage, (*b*) primary membrane formation organizing the first walled stage of the proembryo to show an upper *open* tier and an embryo group below, and (*c*) the *internal* division affecting all nuclei and producing from the embryo group the group of binucleate *E* cells and, by division of the *open* tier, the *upper* tier *U*, commonly ephemeral, and the middle *suspensor* tier, *S*.

The internal division has been definitely shown to occur also in *P. andinus* (Looby and Doyle, 1944*b*), *P. macrophyllus* (Tahara, 1941), *P. nagi* (Tahara, 1941) and *Pherosphaera hookeriana* (Elliot 1950). In other cases published figures of proembryos, often older with suspensors elongating, show, as well as the binucleate *E* cells below the suspensors, a tier of cells above, usually open and with walls coincident with those of the suspensors. This is shown, for example, for *P. dacrydioides* (Buchholz, 1941, Fig. 1), *P. spicatus* (Buchholz, 1936, Fig. 2) and *Saxe-Gothaea* (Doyle and Looby, 1939, Fig. 1 A). It is reasonable to infer that the uppermost tier in these cases is the same tier, derived in the same way, as the *upper* tier, properly so-called, in the first group. In most other published drawings (*cf.* other figures in Buchholz, 1933, 1936, 1941) no upper tier is drawn, nor is it to be expected, as such drawings are mostly of advancing proembryos and the upper tier is commonly ephemeral; but no record clashes with the view that proembryo development in the Family follows a uniform plan. The binucleate condition of the *E* cells has now been shown in all podocarps except *Acmopyle*, of whose development nothing is known, and *Microcachrys*, but in both of these its occurrence may be safely predicted. The production of binucleate cells by the internal division as it affects the embryo cells is a feature apparently limited to podocarps, but, apart from this special feature, the general plan of development is probably basal in all conifers except perhaps the araucarians. The differing embryogenies in the conifers and taxads are but variants of a basal plan showing the same three stages producing similar three-tiered proembryos with an upper tier *U*, a middle suspensor tier *S*, and the basal embryo group.

It is hoped elsewhere to expand this thesis with special reference to embryogeny and polyembryony in *Pinus* but here a few words on nomenclature, of a preliminary nature only, seem advisable. (*a*) It is clearly necessary in description to distinguish between the tier of open cells at first membrane formation and the top tier of cells, also commonly open, at the final three-tiered stage. In the paper on *P. andinus* (Looby and Doyle, 1944*b*), in which its development was fully and clearly worked out, the open tier at the earlier two-tiered stage was called the upper tier, and that at the three-tiered stage the open tier. Since the cells of this latter tier may become completely closed, at least in other cases such as *Cephalotaxus* (Coker, 1907) if not so often in podocarps, this tier cannot *in general for all conifers* be called an open

tier. It is here therefore now called the *upper* tier and the second tier at the two-tiered stage is now called the *open* tier. (*b*) A distinction is needed to avoid confusion between the uninucleate cells of the embryo group at the two-tiered stage and the binucleate embryo cells after the internal division at the final three-tiered stage. Elliot (1950) suggests *primary embryo units* for these cells at the first stage and *binucleate embryo units* for the second stage. Wherever cleavage occurs the term *unit* is probably applicable but if used *in general* it implies that such cells are by nature independent units, which they are not. To avoid implications, which might be argued, it seems better to use the simpler terms, primary embryo *cells* and binucleate embryo *cells* respectively. In these papers the latter are shortly referred to as the *E* cells. Thus at first wall formation the proembryo shows the *open* tier above and the *primary embryo cells* below. At the final three-tiered stage the proembryo shows in succession the *upper* tier *U* above, the *suspensor* tier *S* in the middle and the *binucleate embryo cells E* below. (*c*) Buchholz (1933, 1936, 1941) refers to the elongating cells in the proembryos of podocarps as *pro*-suspensors. It is hoped to show elsewhere that there is no justification for this usage, and here they are simply called suspensors. (*d*) The *upper* tier, here so commonly ephemeral, has been frequently referred to as the ' rosette ' tier. Thus Coker (1902), Sinnott (1913), and Tahara (1941) employ this usage although the term was first used by Mirbel and Spach (1843) for the entirely different tier in the proembryo of *Pinus*. Elliot (1950) even uses the term ' rosette ' in reference to small cell clumps occasionally found in the archegonium of *Pherosphaera* above the basal plug and claimed by him to be derived from haploid nuclei introduced by the pollen tube. Buchholz (1941), recognizing a morphological difference between the ' rosette ' of *Pinus* and the ' rosette,' in Coker's sense, of a podocarp, but without apparently appreciating the real nature of the developmental difference, seeks to confine the term to the occasionally occurring small *walled* cells above the suspensors, and seeks in podocarps for such *walled* cells to compare with the walled ' rosette ' tier of *Pinus*. The double cell in the ' rosette position ' in *P. urbanii* (Fig. 11), the single small cell in *P. macrophyllus* (Fig. 19), the three small isolated cells in *P. nankoensis* (Fig. 42), and the single elongated cell in *Phyllocladus alpinus* (Fig. 45) are examples of the sort of ' rosette ' cells sought for and described by him. Looby and Doyle (1944*b*) also illustrate two such cells in *P. andinus* (Fig. 66), but on the whole such cells are rare in podocarps though often occurring much more prominently in other conifers. They do not, however, form a properly organised tier and may have quite different origins. Thus the cells of what is here called the *upper* tier, typically open to the archegonium above, may, some or all, become fully closed. *Cephalotaxus* (Coker, 1907) is a notable example from another group. Some too of the original *S* cells may not elongate and may appear later as small cells above the main suspensors. It is possible too, but this has still to be confirmed, that closed *upper* cells may proliferate slightly, or even, rarely, that cells may be cut off from the tops of the suspensor cells when young. In any single case of an advanced embryo showing such cells it would be hard indeed to decide on their real origin. But such sporadic occurrences of mixed origin do not constitute a ' rosette ' in any way morphologically equivalent to the clean-cut ' rosette ' tier in *Pinus*. The term ' rosette ' cells should not be applied to

them, and is not here used. The questions arising are among those to be discussed elsewhere, so it must suffice to state that least of all can the term ' rosette ' be used to designate what is here called the *upper* tier. The *upper* tier, and in the earlier phase the *open* tier, are both formal components of proembryo development. Buchholz in his search for walled ' rosette ' cells gives no importance to this *upper* tier, perhaps because ordinarily it is so ephemeral, and, when it is clearly obvious in some of his preparations, adds to the confusion by dismissing it abruptly as composed of relict nuclei (*cf.* 1941, his account of *P. dacrydioides*, p. 4 and Fig. 1). The *upper* tier is a formal product of the internal division, and the term *relict* nuclei has here been specifically kept for those diploid nuclei extruded from the embryonic cytoplasm during the free nuclear phase.

Apart from variations, arising from such extrusion, in the number of nuclei incorporated in the primary walled stage of the proembryo two principal modifications of the basal plan occur in the Family. These are concerned respectively with the free mitoses before wall formation, and with, later, the number and arrangement of the *E* cells.

(*B*) *The free mitoses.*

The contrast between *P. nivalis* (Boyle and Doyle, 1954) and *P. andinus* (Looby and Doyle, 1944*b*) illustrate the problem. *P. andinus* has 5 free mitoses, of which 2 occur above in the archegonium, more or less in the position of the fusion nucleus. *P. nivalis* has 4 free mitoses, of which only 1 occurs above. The question concerns the distribution of these phenomena and their possible basal nature in the Family.

Most frequently there are 4 free mitoses yielding a maximum of 16 free nuclei before membrane formation. This has been definitely shown only for *P. nivalis* (Boyle and Doyle 1954), *Saxe-Gothaea* (Looby and Doyle, 1939) and *Pherosphaera hookeriana* (Elliot, 1950), but, although the actual mitoses have not been demonstrated, it is almost certainly the condition in all typical eupodocarps (Boyle and Doyle, 1954), in the *Dacrycarpus* section of *Podocarpus*, in *Dacrydium*, in *Phyllocladus*, (Buchholz, 1933, 1941), and in *Microcachrys* (Lawson 1923). But within *Podocarpus* 5 free mitoses, yielding a possible 32 free nuclei, occur in the sections *Stachycarpus* and *Nageia*. The 5 mitoses have been demonstrated for *P. andinus* (Looby and Doyle, 1944b) and *P. nagi* (Tahara 1941) and must be accepted for *P. usumbarensis* (Buchholz, 1936), *P. gracilior*, *P. amarus*, *P. blumei*, and *P. nankoensis* (Buchholz, 1941). In these latter the earliest stages available have suspensors elongating, but as these range in number from 18 to 23 with usually more than 8 *E* cells there must have been at least 5 mitoses. There is however some doubt about the conditions in *P. spicatus* and *P. ferrugineus* in which only 4 mitoses, apparently assumed and not seen, are reported by Sinnott (1913). Buchholz (1936) is indefinite on the point. It seems on a cursory view a little anomalous that a lower member of *Stachycarpus* like *P. andinus* and a higher member like *P. amarus* should show 5 mitoses while intermediate forms like *P. spicatus* and *P. ferrugineus* show only 4. Re-examination of the available evidence however suggests that the occurrence of 5 mitoses in these

forms cannot be ruled out. Buchholz (1936), in his dissected embryos of *P. spicatus* in which the *E* cells cannot have increased in number from the primary stage as they are still binucleate, gives the *S* : *E* ranges as 7–9 : 9–12. On these numbers most embryos must have incorporated more than 16 primary nuclei, so that more than 4 mitoses must have been common. Obviously with 5 mitoses, if, say, 2 nuclei were extruded after the third mitosis and another 2 after the fourth mitosis, the resulting total of available free nuclei might only be 20, so that the numbers recorded by Buchholz could readily follow from 5 mitoses and associated extrusion. This is supported by Sinnott's (1913) record of $S = 9$ for *P. spicatus*, though the suspensor number is probably more variable. Sinnott's account of the behaviour of the *E* cells however is not easy to interpret. As in *P. andinus* the embryo group in *P. spicatus* shows a large terminal cell and a number of smaller cells between it and the suspensors. This group is reported by Sinnott to be small at first, but its members are said to divide immediately and to number from 20 to 40 before the suspensors begin to elongate actively. There seems to be some faulty interpretation here. It clashes with the established records that, in podocarps, the *E* cells, after the internal division giving the binucleate condition, remain quiescent till suspensor elongation is practically complete. However, given 5 mitoses and a variable degree of extrusion, the total cell number in proembryos of *P. spicatus* might be expected to vary considerably, and those with a higher *E* number would be, not older ones, but ones derived from a free nuclear condition in which all or most of the possible 32 nuclei were available at primary wall formation. There is, however, no evidence given to support the occurrence of up to 40 *E* cells. Unless *P. spicatus* is a complete exception to typical development in *Podocarpus*, and from Buchholz's account and figures it is not, an embryo group of 40 cells could only arise as a rare case such as the one recorded for *P. andinus* in which a 6th mitosis must have taken place. Sinnott's figure (Pl. 9 Fig. 46), given in support, could not, by comparison with the example from *P. andinus* (Looby and Doyle, Pl. 14, Fig. 67), be such a case. This figure of Sinnott's, which is the only reference given in support of the occurrence of up to 40 cells, does not permit an accurate count, and shows no more in this one section photographed than can be found typically in corresponding stages of *P. andinus*. The figure, a low power photograph, is hard to follow in detail, but some of the cells seem to be binucleate, which should of course be the condition at this stage. Even when familiar with a wide range of material it is seldom easy to count accurately the cell number from one section on a slide, and unless the occurrence of paired nuclei in *all* these cells was appreciated very high numbers might be guessed at. Sinnott thus most probably simply misinterpreted his material, but his account in general emphatically supports the occurrence of 5 mitoses in *P. spicatus*, giving a maximum of 32 nuclei rather than 16 derived from 4 divisions.

For *P. ferrugineus* Buchholz (1936) gives no record of *S* number in his descriptive section, only entering 7–9 in a diagram in the later paper (1941, Fig. 51), an entry apparently simply copied from its relative *P. spicatus*. From what is now known about variation in *P. nivalis* the range in *P. ferrugineus* could easily exceed this 7–9 estimate. He records, however, up to 10 *E* cells. Sinnott (1913), too, gives no record of *S* number, stating merely that there are rarely more than 8–12 *E* cells

until the embryo group has pushed far into the gametophyte tissue. The supposed early division in the *E* group claimed for *P. spicatus* before active *S* elongation is stated not to occur, a statement in this case fully substantiated by his Fig. 47, Pl. 9. The *E* cells in *P. ferrugineus* clearly remain typically quiescent during suspensor elongation rendering the claim for early multiplication of *E* cells in *P. spicatus* all the more doubtful. Data however for *P. ferrugineus* are still meagre, but if cases of 12 *E* do occur these, even if associated with only a minimum 7 *S*, would give 19 effective free nuclei at primary wall formation, a number necessitating more than 4 mitoses.

This scanty evidence seems to show that in *P. spicatus* 5 mitoses must have occurred, and that such is possible, perhaps even probable, for *P. ferrugineus*. If so, pending significant proof, it could be taken that the whole *Stachycarpus* section is characterized by 5 free mitoses, a feature definitely shared by the section *Nageia*. The dropping of one mitosis in *P. ferrugineus* cannot however be ruled out, and such a dropping out, if confirmed, would be of some interest, showing that such a change could occur within a narrow affinity range.

The question then whether 5 or 4 free mitoses are to be considered basal in the Family, like most other biological questions, can only receive a probable answer, but on *a priori* grounds the larger number could be so considered. If the embryogeny of *Ginkgo* is as conservative as its fertilization then 6 free mitoses or more may have been a feature of early conifers, and, in members of the group, a larger number of free nuclei would represent a more conservative condition, a lesser number, due to the dropping out of one or more of the mitoses, a more advanced condition. Throughout the conifers there seems to be a tendency towards shortening the free nuclear phase, a tendency inherent in conifer evolution. Thus in the primitive *Ginkgo* there are 8 free mitoses ; in the Araucariaceae probably 6 ; in *Sciadopitys* and the simpler *Podocarpus* sections, *Stachycarpus* and *Nageia*, 5 ; in most podocarps, including all the higher ones, 4, a number shared by most taxads and *Cephalotaxus* ; and in the Pinaceae, Taxodiaceae, and Cupressaceae normally 3. Within the latter two families and the Taxaceae further special reductions are shown. Thus in the Taxaceae *Torreya*, commonly recognized as a more advanced form than say *Austrotaxus*, has, instead of the 4 typical of the Family, only 2 free mitoses (rarely 3), giving normally a 4-celled proembryo. Similarly in the Cupressaceae the number is likewise reduced to 2 instead of 3 in the southern specialized callitroids, a change paralleled in the Taxodiaceae in the southern *Athrotaxis*, in both cases again producing a 4-celled proembryo. The extreme of this tendency is shown of course by *Sequoia sempervirens*, in which wall formation follows directly on the first mitosis, a phenomenon which may also be seen, but as a rare occurrence, in *Athrotaxis*. A *decrease* in the number of free mitoses seems thus associated, at least roughly, with taxonomic advance, and in no group is an *increase* in mitotic number so related. On such grounds 5 free nuclei could by choice be considered more primitive in podocarps. This condition would thus have been retained in *Podocarpus* in the *Stachycarpus* section, whose members are commonly considered the more primitive in the genus, and in the hardly less primitive *Nageia* section. The higher *Podocarpus* species and the rest

of the Family, many of whom like *Pherosphaera* clearly exhibit features of specialization or advance, would thus show a general expression of this common conifer tendency towards shortening of the free nuclear phase. Hence the demonstration of this tendency within *Stachycarpus*, if confirmed for *P. ferrugineus* though such is not in fact expected, would hold some special interest.

Another feature of early embryogeny bears indirectly on this point, a feature in which *P. andinus* and *P. nivalis* again stand in contrast. In *P. andinus* (Looby and Doyle, 1944*b*) and in *P. spicatus* (Sinnott, 1913) the fusion nucleus in the archegonium divides, more or less *in situ*, twice, the 4 resulting nuclei passing to the base, and, since the other investigated species of *Stachycarpus*, with the doubtful exception of *P. ferrugineus*, agree with *P. andinus* in having 5 free mitoses, it is probable that this behaviour, shown by *P. andinus*, is typical of the section. In *P. nivalis*, in contrast, there is only one division *in situ*, with 2 nuclei passing to the base, and it has been shown in a recent survey (Boyle and Doyle, 1954) that this condition is equally characteristic of the whole *Eupodocarpus* section. Unfortunately the distribution among the genera of these two conditions is another of the podocarpean features on which the date are still inadequate. Thus, keeping to *Podocarpus*, the condition in the *Nageia* section is uncertain. The only available statement on the point seems to be the ambiguous one by Tahara (1941) for *P. nagi*. " After fertilization the fusion nucleus divides into two. They still remain in the middle part of the archegonium. But the nuclei in the next four nucleus stages are found at the bottom of the archegonium. The eight, sixteen and thirty-two nucleus stages come next ". By inference from the last sentence the " next four nucleus stages " must mean " the succeeding 4-nucleate stage " but even this emendment still leaves some uncertainty as to the location of the second mitosis. Since, however, it is stated so clearly that the two nuclei from the first division remain in position Tahara seems to suggest that a second division follows *in situ* but was not actually seen. Provisionally that interpretation is the one here accepted, partly because 5 free mitoses are characteristic of the section. In contrast, in the *Dacrycarpus* section, with only 4 free mitoses, Sinnott (1913) definitely records only 1 mitosis *in situ* for *P. dacrydioides*, which thus agrees on both points with *Eupodocarpus*. *P. dacrydioides* (Buchholz, 1941) also agrees with *Eupodocarpus* in other points which seem to be points of advance, such as the reduction of the *E* cells to a small number in one plane (*v. infra*). Thus within *Podocarpus* the tendency shown by the free nuclear phase seems to be a transition from 5 mitoses with 2 *in situ*, exhibited by the simpler *Stachycarpus* and *Nageia* sections, to 4 mitoses with only 1 *in situ* in the more advanced sections. This too is the condition in the specialized *Pherosphaera* (Elliot, 1950) and probably also in *Dacrydium cupressinum* (Sinnott, 1913). Since the later embryogeny of *D. cupressinum* is certainly of a rather advanced type (cf. Buchholz, 1933), related thus to the advanced transition in the free nuclear phase, it is suggested that other species of *Dacrydium* might repay investigation. It is possible that less advanced species in the genus might show 2 mitoses *in situ*, the contrast between *P. andinus* and *P. nivalis* being repeated.

The situation in *Microcachrys* is quite unknown, the earliest post-fertilization stage reported (Lawson, 1923) being that of the late mature proembryo with elongating suspensors.

Although there are three papers on *Phyllocladus* (Kildahl, 1908 ; Young, 1910 ; Holloway, 1937) only Kildahl records any early divisions, and actually draws the second sporophytic mitosis. It is however impossible, from script or illustration, to come to any decision on its location.

In contrast in *Saxe-Gothaea* (Looby and Doyle, 1939) there are definitely 2 divisions of the fusion nucleus more or less *in situ*. This record calls for special comment. In the first paper of this series (Boyle and Doyle, 1953) it was suggested that certain features in *Stachycarpus*, notably the deep early embedding of the archegonium and the gross inequality of the fusing nuclei at fertilization, were probably special developments in the section. It was thus possible that the occurrence of 2 sporophytic mitoses above was but another special development in the section. The certain occurrence however of 2 mitoses *in situ* in *Saxe-Gothaea*, and their probable occurrence in *Nageia*, suggests that this feature is something more than a special development in *Stachycarpus*, especially as *Saxe-Gothaea* shows many other features in its gametophytes and embryogeny which seem generalized and basal rather than advanced (*cf.* Boyle and Doyle, 1953, and later in this paper). It is of interest also in this connection that in *Sciadopitys* (Tahara, 1937), a form with an undoubtedly primitive type of proembryo with 5 free mitoses, the first two divisions occur above, and, in *Cephalotaxus* (Lawson, 1907 ; Coker, 1907), whose embryogeny is also rather primitive, the two nuclei derived from the first division apparently carry out the second mitosis on their passage down to the archegonial tip.

It seems legitimate to conclude, even if only provisionally, (1) that the basal podocarp had 5, or perhaps even 6, free embryonic mitoses, of which at least 2 occurred above in the archegonium in the position of the fusion nucleus ; (2) that this condition is retained by the simpler *Stachycarpus* and *Nageia* sections ; (3) that *Eupodocarpus* and the other modern genera exhibit a tendency, common in conifer evolution, to reduce the number of free mitoses, in this case to 4 ; and (4) that this tendency is accompanied by the other tendency to postpone the second sporophytic mitosis to the base of the archegonium, a tendency to which *Saxe-Gothaea*, with perhaps a few other forms yet to be determined, is a definite exception.

(*C*) *Number and arrangement of* E *cells.*

Here too the contrast is shown by *Podocarpus andinus* with up to 15 *E* cells in a complex or storied arrangement and *P. nivalis* with only 1–3 *E* cells in a single tier.

The number of free nuclei available for incorporation into the first walled stage of the proembryo is of course determined by the number of free mitoses and the number of extruded *relict* nuclei, but otherwise the number of cells in the *open* tier seems to be determined by the *E* cells, which vary much in number and particularly in arrangement but which show definite trends. It is as if the nuclei remaining after

the organization of the embryo group became arranged in one plane to form an *open* tier.

It was shown in the account of embryogeny in *Podocarpus nivalis* (Boyle and Doyle, 1954) that the *E* number was not constant, even for proembryos formed by the one individual plant, but varied over a range, in this case the narrow range of 1–3. Variation of *E* numbers over a range seems to be a feature of podocarpean embryogeny, so that in comparing *E* numbers in the sections or groups of species it is the ranges that are to be compared. In Table 1 the *E* numbers with the accompanying *S* numbers, expressed as *S* : *E*, are given as far as at present known, the *S* numbers being included simply to give a better picture of the proembryo. For reasons given in the paper on the embryogeny of *P. nivalis* certain of these numbers, at any rate the *S* numbers, may be only approximate, though the *E* numbers are probably more reliable. As the *Eupodocarpus* species have already been considered and compared (Boyle and Doyle, 1954) this table does not include each by name but gives the general result for the section.

TABLE I.

S : *E numbers in podocarps.*

	S	*E*	
A.			
Podocarpus andinus ...	9–13	11–15	Looby & Doyle, 1944 *b*
P. spicatus	6– 9	9–12	Buchholz, 1936
P. ferrugineus	7– 9 (?)	7–10	Buchholz, 1936
P. usumbarensis ...	18–23	9–11	Buchholz, 1936, 1941
P. gracilior	about 20	about 10	Buchholz, 1941
P. amarus	about 20	about 10 (?)	Buchholz, 1941
P. Nagi	23–25	7– 9	Tahara, 1941
P. nankoensis	19–20	10–11	Buchholz, 1941
B.			
Podocarpus dacrydioides ...	8–10	3– 6	Buchholz, 1941
P. imbricatus	9–10	3– 4	Buchholz, 1941
Eupodocarpus sp. ...	8–14	1– 3	Boyle & Doyle, 1954
Pherosphaera hookeriana ...	6–10	(2) 4– 6 (9)	Elliot, 1948, 1950
Dacrydium cupressinum ...	7–11	5– 7 (9)	Buchholz, 1933
Saxe-Gothaea conspicua ...	3– 4	10–12	Looby & Doyle, 1939
Phyllocladus alpinus ...	4– 6	9–12	Buchholz, 1941

Group A have 5 free mitoses (except perhaps *P. ferrugineus*).
Group B have 4 free mitoses.

In the account of embryogeny in *Podocarpus nivalis* it was also shown that the range of *S* and *E* numbers was much wider than would have been expected from previous *Eupodocarpus* records. These differed from one another but all actually

fell within the *P. nivalis* range. Examination of a large number of embryos of the other species would almost certainly have somewhat extended the range of each. So pending a knowledge, comparable to that established for *P. nivalis*, of the *S* and *E* range of many more species it would be inadvisable to read too much into these figures. For instance, the relatively greater *S* numbers in the higher stachycarpean species and their agreement with the *S* numbers of the *Nageia* species, in contrast to the lesser numbers in the lower *Stachycarpus* species resulting presumable from the extrusion of more embryonic nuclei at the free phase in these latter, might well be of interest and even of taxonomic importance but would need confirmation.

Two points however do appear established. The *E* numbers in *Saxe-Gothaea* and *Phyllocladus*, though these have only 4 free mitoses, are the same, within limits, as in the species of *Stachycarpus* and *Nageia* with 5 free mitoses in their early embryogeny. The *S* numbers in the former genera are then correspondingly low. It is suggested that this is significant and that it indicates that the primitive podocarps had a large number of primary embryo cells, perhaps even more than are found in *P. andinus*. In the first paper of this series (Boyle and Doyle, 1953) evidence was adduced that the gametophytes of *Saxe-Gothaea* probably resembled, possibly even closely, the basal podocarpean type. It is of interest that in *E* cell number its proembryo too seems basal and primitive.

The other point appears most clearly in the genus *Podocarpus*, within which is shown a clear tendency towards the development of a smaller number of *E* cells. This is most marked in the advanced *Eupodocarpus* section, in which the number does not exceed 3, is most commonly 2, and in certain instances, as in *P. macrophyllus*, may even be reduced to 1 in the majority of embryos, the greatest possible expression of this tendency. The same tendency is clearly expressed in the *Dacrycarpus* section as shown by the low numbers in *P. dacrydioides* and *P. imbricatus*. It is expressed too in *Dacrydium* and *Pherosphaera* though not so strikingly. In the latter, according to Elliot (1950), the number may be as low as 2 and as high as 9. No formal estimate is given of the relative frequencies of the extreme numbers, but numbers such as 8 or 9 seem from the context to be exceptional, and Elliot states definitely that 3 or 4 are by far the commonest. *Pherosphaera*, being a rather specialized type, might be expected to show signs of advance, and a range of *E* numbers such as 3–4 would appear more in keeping with its status. However, as nothing is known of the factors determining the adoption of small *E* numbers in podocarpean proembryos, *Pherosphaera* must be left as a form in which the tendency to the reduction of *E* numbers is commonly expressed but in which the determining factors, on occasion, are not fully effective. *Dacrydium* seems to show a rather similar condition. The range given for *D. cupressinum*, 5–7 with an occasional extension to 9, is clearly lower than the range in *Saxe-Gothaea* and *P. andinus* or *Phyllocladus* and suggests a certain degree of advance from the basal type, with again an occasional embryo in which the determining of the tendency is not fully effective. But information on other *Dacrydium* species is urgently needed to confirm or refute the possibility that other species may more closely resemble the *Phyllocladus-Saxe-Gothaea* type. The information available for *Microcachrys* (Lawson, 1923) is too indefinite to be

of real value in this connection, but the figures given by Lawson suggest a rather wide range of *E* numbers, so that some embryos could be as primitive as *Phyllocladus* and others as advanced as *Dacrydium*.

It is suggested therefore in this connection that basally in podocarps the *E* number was high; that this high or relatively high number has been retained in *Stachycarpus*, particularly the simpler members, in *Nageia*, in *Saxe-Gothaea*, in *Phyllocladus*, and perhaps in some forms of *Microcachrys;* and that the Family shows a tendency towards the reduction of the *E* number, a tendency very evident in *Dacrycarpus*, particularly so in *Eupodocarpus*, and a little less marked in *Dacrydium* and *Pherosphaera*.

The arrangement of the *E* cells is perhaps of greater interest. In certain types the *E* group is markedly tiered or storied showing a terminal cell with a group of cells arranged very roughly in three to five rows between it and the suspensors. This is best shown in *Saxe-Gothaea* (Doyle and Looby, 1939, Fig. 1A) *Podocarpus andinus* (Looby and Doyle, 1944 *b*, Fig. 41), *P. spicatus* (Buchholz, 1936, Figs. 1–3), *P. ferrugineus* (Buchholz, 1936, Figs. 13, 14) and *Phyllocladus alpinus* (Buchholz, 1941, Fig. 43–45; Holloway, 1937, Figs. 36, 39). In the first three, especially in *P. andinus*, the terminal cell is particularly large and distinct and in *P. andinus* and *P. spicatus* is apparently not incorporated in the later embryonic meristematic mass. Marked tiering is thus shown in three distinct podocarpean lines. Within *Podocarpus* a tendency is shown for the *E* cells to be arranged, irrespective of their number, in a horizontal group in which all, or nearly all, the *E* cells are in direct contact with the S cells. This is most clearly exhibited again in the advanced *Eupodocarpus* in which the tendency to reduction in *E* cell number is most fully expressed. In this group, when 2 or 3 *E* cells are present, these cells lie in one plane tier. In *Dacrycarpus*, too, as illustrated by *P. dacrydiodes* (Buchholz, 1941, Figs. 1–6), the *E* cells, relatively few, 3–6, are likewise arranged in a single tier. But even in the higher members of the *Stachycarpus* section, where as many as 11 *E* cells may occur, as many as in the storied groups of *Saxe-Gothaea* or *P. spicatus*, the *E* cells are also grouped in one horizontal row. This is known for *P. usumbarensis* (Buchholz, 1936, Figs. 19–21) and *P. gracilior* (Buchholz, 1941, Fig. 41), and may occur also in *P. amarus*. Contact of each one of so many *E* cells with the suspensor group seems to be made possible by the occurrence in these species of a large number of *S* cells (Table 1). Essentially the same development is seen in the *Nageia* section as illustrated by *P. nagi* (Tahara, 1941, Text-fig. 10) and *P. nankoensis* (Buchholz, 1941, Fig. 42). In these *Nageia* species however there is the minor difference from the similar *Stachycarpus* ones that the main group of large *E* cells surrounds two small cells hidden in the centre, so that a couple of the larger cells apparently do not make direct contact with the suspensors, a condition which may represent a very partial retention of a storied arrangement.

It is suggested here that the storied arrangement is more primitive, and that the arrangement of cells in one tier is derivative.

This is quite counter to the views of Buchholz (1941). Although stating that he considered *P. usumbarensis* and *P. nagi* to be more advanced taxonomically

than *P. spicatus* or *P. andinus* he nevertheless claimed that the former had kept a more primitive embryonic condition. In general the organization of many *E* cells in a *single* tier was to be considered more basal, and from this condition two developments sprang, illustrated by the diagrams in his Figs. 51 and 52. One was characterized by a transition from 5 free mitoses to 4 and by a reduction in *E* number leading through *P. dacrydioides* to the *Eupodocarpus* condition. Incidentally though *Dacrycarpus* and *Eupodocarpus*, whatever their origin, must of course be recognized as characterised by a reduction in *E* cell number, the suggested separation of the investigated *Eupodocarpus* species into separate blocks is probably of little value. Buchholz groups separately, *P. urbanii* and *P. glomeratus* with $S : E = 11–13 : 2–3$, *P. macrophyllus* with $S : E = 14–15 : 1–2$, and *P. totara* and *P. nivalis* with $S : E = 7–10 : 1–2$. But as the actual demonstrated range in *P. nivalis* (Boyle and Doyle, 1954) is $S : E = 8–14 : 1–3$, no distinction on embryogeny can thus be made in *Eupodcarpus* on present knowledge. The other line, also presumed to be characterized by a transition from 5 free mitoses to 4, led to the development of an increasing storied arrangement through forms like *Dacrydium* and *Podocarpus ferrugineus* to types like *P. spicatus*, *Phyllocladus*, and *Saxe-Gothaea*. A storied arrangement of *E* cells was thus considered derived, and was claimed to be organized in relation to the long narrow tip of the archegonia shown by these latter types. Now Buchholz in all his writings makes embryogeny a character of fundamental importance in establishing relationships, and in this case looks on the series given in his Figs. 51 and 52 as something more than merely a series diagrammatically representative of possible development tendencies. " Taxonomists " he says " who may contemplate a monographic treatment of this family should search for external criteria, correlated with the series shown by embryogeny, as a guide to an improved scheme of phylogeny ". This is not the place to discuss the value of embryogeny as a criterion in taxonomy, even if fuller data on embryogeny were available than that gained from the cursory examination of a few older embryos from a couple of ovules. Here, however, Buchholz's emphasis on the overweening importance of embryogeny in taxonomy is not accepted, either in general or as applied to podocarps in particular. In the present state of our knowledge, in particular in view of the paucity of our knowledge of so many podocarps, if, on general grounds, A and B appear related because of similarities in distribution, vegetative structure, strobilus organization, and the like, then a similarity in embryogeny might be adduced in confirmation. That is as far as it is safe to go. But it is this insistence on the importance of embryogeny which lends interest to considering whether Buchholz's series is in fact a probable series, even if embryogeny had all the value claimed for it.

Ignoring then all the taxonomic implications, and considering the question as simply a question in the embryogeny of the podocarps, the more probable view would seem to be that the storied arrangement of the *E* cells is the more primitive, and that their arrangement in one tier is derived.

At the time when Buchholz drew up his series the *P. usumbarensis–P. nagi* group was known to have 5 free mitoses in early embryogeny, *Saxe-Gothaea* was known to have 4 only, and 4 only were also presumed for *P. spicatus*. *P. andinus*

had not been investigated. It is now known that *P. andinus* has definitely 5 mitoses (Looby and Doyle, 1944 b), and it is all but certain, only awaiting confirmation, that *P. spicatus* has also 5 (*v. supra*). So, with the very doubtful exception of *P. ferrugineus*, the occurrence of 5 free mitoses is characteristic of all stachycarpean species examined, and is not a feature limited to the higher forms only. The argument that *P. spicatus* is derived therefore, as far as it is based on a reduced number of free mitoses, loses its force.

There would still, of course, be no *a priori* reason to maintain, because *P. andinus* and *P. spicatus* were taxonomically at a lower level than, say, *P. usumbarensis*, that their *embryogenies* must be more primitive. Within *Stachycarpus*, *P. usumbarensis* could well have retained a primitive embryogeny coupled with advancing strobiloid features, *P. andinus* and *P. spicatus* reversing the development. The similarities however in female gametophyte and embryogeny between *P. andinus* or *P. spicatus* and *Saxe-Gothaea*, similarities which practically amount to identity except for the loss of one free mitosis in the latter, are so remarkable that they must be significant. They have however been sufficiently summarized elsewhere (Looby and Doyle, 1944 *a* ; Boyle and Doyle, 1953), and need no further elaboration here except to emphasize the storied arrangement of the *E* cells common to both. Comparison with other forms makes it difficult to establish a necessary relationship between this storied arrangement and long pointed archegonia. *Phyllocladus*, representative of a line which must have been long distinct from the *Saxe-Gothaea* line or the primitive species of *Podocarpus*, shows a similar storied arrangement differing from the other forms only in the lesser dominance of the terminal *E* cell which resembles its fellows except in position ; yet its archegonia are certainly shorter and less pointed. Elsewhere too than in podocarps similar storied embryos occur in types which on general grounds appear relatively primitive. *Austrotaxus spicata*, for instance, with archegonia no longer or more pointed than those in an advanced podocarp like *P. nivalis*, has a proembryo (Saxton, 1943, Figs. 14, 16), derived from 4 free mitoses, the storied arrangement of whose embryo cells, even down to the single dominant terminal cell, remarkably resembles that in *Saxe-Gothaea*. In *Cephalotaxus* (Coker, 1907), likewise, the embryo cells are storied in a manner as narrow and as longdrawn out as in *P. andinus*, with a terminal cell, or even cells, which is nonfunctioning. Significant too is a similar marked storied arrangement in a form as remote from any of these as *Sciadopitys* (Tahara, 1937). The balance of probability seems to favour the concept that the primitive conifer embryo was a relatively massive multicellular group, a condition represented in a number of more primitive living forms by the still somewhat massive storied primary embryo, and that the higher conifers in general showed tendencies to a reduction in number of the embryo cells and their arrangement in a single tier. The extreme dominance, in *P. andinus* and *P. spicatus*, of the terminal cell, which is not incorporated into the maturing embryo and which may function like a boring tip during suspensor growth, may be a special feature developed in the lower stachycarpean species ; and *this* special feature, but not the general storied arrangement, *may* be related to the long narrow tip of the archegonium in such species. If so, the *E* group, basal in the podocarps,

might well have shown an organization somewhat between *Saxe-Gothaea* and *Phyllocladus*.

The acceptance of such a storied arrangement as primitive in podocarps simplifies comparison of the *E* groups in the whole family. The genera and species then simply show various grades in expression of the common tendency towards the organization of the *E* cells in one tier, the expression of which coincides in most cases with that of the other tendency towards a reduction in number of the *E* cells. Thus, as already noted, the higher species of *Stachycarpus*, retaining a high E number, would have advanced to a condition in which the *E* cells are arranged in a single tier, a development also shown in the *Nageia* group. In *Dacrycarpus* and particularly in *Eupodocarpus* the two tendencies would have been effective, producing only a few *E* cells in one tier. Here may be an example of a close relation between taxonomic advance and embryological advance in so far as the *E* group in *Eupodocarpus* is even smaller and more refined than in *Dacrycarpus*, in which, even if only on occasion, the *E* group may still rise to 6.

The other forms take an intermediate position. As far as can be judged from Lawson's (1923) figures of *Microcachrys* the *E* group is not drawn out into a strongly storied sequence, nor is it in a single tier, but the group, in which the cell number is uncertain, seems to show two more or less equal tiers. The *E* group in *Microcachrys* appears to stand roughly in the same developmental relation to that of the basal *Saxe-Gothaea–Phyllocladus* type as *Taxus* does to *Austrotaxus*, *Taxus* (Sterling 1948, Fig. 10), though its archegonia are small and almost circular, showing two distinct embryo tiers at primary wall formation. *Pherosphaera* (Elliot, 1950) is variable. The common *E* number is 3 or 4 and in such cases the *E* cells lie in a single tier. But the *E* numbers may rise occasionally even up to 9, and with *E* numbers greater than 4 or 5 the *E* cells tend to lie in two tiers, the lower with only one or two of the cells. In this rather specialized form both tendencies are again shown but on occasion neither may be fully effective. *Dacrydium* (Buchholz, 1933) seems to show a condition resembling that in *Pherosphaera* when a larger number of *E* cells develop, but it must always be remembered that there is only available for *Dacrydium* the most meagre data from a few ovules of only one species, *D. cupressinum*. If the genus were as well known as *Podocarpus* the *E* cells might well show quite a wide range in number and arrangement. At any rate as far as has been recorded for *D. cupressinum* the *E* group shows two tiers, the lower with only one or two cells, which are said to be commonly larger than those in the upper tier in contact with the suspensor. As the *E* group number is 5-7, occasionally rising to 9, it is tempting to predict that simpler species of *Dacrydium* might well show an arrangement closer in appearance to the *Microcachrys* type. In any event, on the view here adopted, the embryo group in *Dacrydium* shows a condition of relative advance.

In general then it is suggested that the basal *E* organization in podocarps showed a group of many binucleate cells superimposed in rough tiers somewhat as in the living *Saxe-Gothaea*. Within the Family two tendencies appear, the tendency

to reduction in *E* number and the tendency to the arrangement of the *E* cells in one tier, both tendencies reaching a full expression in *Dacrycarpus* and particularly in *Eupodocarpus*, in which embryos with only one *E* cell are common.

(*D*) *The embryo-tetrad.*

Suspensor growth seems to show no general features of specialization, though there may be minor differences among the species, such as relative *S* length, which are not here being discussed. It is the behaviour of the *E* cells at this and later stages which is here of interest. Conditions at these stages in *Microcachrys* have been left obscure and practically unexamined (Lawson, 1923), and in *Acmopyle* are quite unknown, but otherwise, with the doubtful exception of *Pherosphaera*, which will be mentioned later, all available records report that the *E* cells remain undivided and binucleate during suspensor elongation. This has been reported for *Saxe-Gothaea* (Doyle and Looby, 1939), for *P. andinus* (Looby and Doyle, 1944 *b*), for *P. nivalis* (Boyle and Doyle, 1954), and by Buchholz (1933, 1936, 1941) for *Dacrydium cupressinum*, *Phyllocladus alpinus*, and 14 other species of *Podocarpus* from all sections except *Microcarpus*. Normally on such evidence the conclusion would have been legitimate that such behaviour was a characteristic podocarpean feature, but in this case a difficulty arises. Elliot (1948, 1950) claims for *Pherosphaera hookeriana* that the embryo cells become binucleate in the ordinary way but that " the binucleate cells, instead of remaining binucleate, immediately proceed to form two-celled units ; the binucleate cell phase is of very short duration ". The two-celled units are claimed to remain as such during elongation.

This claim of Elliot's raises acutely the question of the occurrence throughout the podocarps of the *embryo-tetrad* stage, and necessitates a reconsideration of the point.

The problem is to determine how and at what stage the binucleate *E* cells produce the later uninucleate condition, a problem first clearly visualized by Buchholz (1936). In that paper, dealing with embryogeny in some stachycarpean types, he had suggested briefly, in connection with *P. spicatus*, that " ultimately cell plates appear between the two nuclei of each embryo initial or unit followed by other cell divisions ". *Ultimately* refers to the fact earlier noted by him that the embryonic cells remain binucleate for a considerable period during suspensor elongation. Doyle and Looby, influenced by this statement by Buchholz, later (1939) stated for *Saxe-Gothaea* that " eventually a membrane forms between each of the pairs of nuclei " and even gave a figure (Fig. 3 G) to illustrate the condition. It will be noted that these two records, if substantiated, would have meant that the behaviour in the two types concerned would be that claimed by Elliot for *Pherosphaera* except that in the latter there is the additional peculiarity claimed that such membrane formation occurs before suspensor elongation. These earlier records by Buchholz and by Doyle and Looby were however based on the most meagre material, and grave doubt arose about their accuracy when Buchholz (1941) recorded in one dissected embryo

of *Podocarpus totara* a double mitosis in one *E* cell and a tetrad arrangement of uninucleate cells replacing the other *E* cell of the embryo. Recognizing further the occurrence in other species of uninucleate cells in groups of 4 at the suspensor tips he drew the wide inference that what is here called the *embryo-tetrad* stage was a characteristic feature, not only of *Eupodocarpus* in which it was first recorded, but of all podocarps. " It is probable that in all podocarps the binucleate cells directly form similar 4-celled groups " (Buchholz, 1941, p. 16). So broad an inference from such slender evidence needed confirmation. Such confirmation was given a little later in *P. andinus*, perhaps the simplest living *Podocarpus* species, by Looby and Doyle (1944 *b*) who also showed that after the double mitosis in the binucleate *E* cells the 4 nuclei lay free in the original *E* cell within which membranes were then deposited between them to give the tetrad of uninucleate cells. While work on *P. nivalis* was still in progress Elliot's papers (1948, 1950) on *Pherosphaera* appeared containing the claim for the immediate formation, even before suspensor elongation, of 2-celled units from the binucleate *E* cells by simple membrane formation between the nuclei, the method originally considered and then rejected on evidence both by Buchholz and by Looby and Doyle. It is clear of course that the mere grouping of embryonic uninucleate cells in fours is no proof for Buchholz's thesis. Two-celled units, as conceived by Elliot, in each of which another division had occurred, would equally give a tetrad appearance. The valid proof is the demonstration of the double mitosis. Also it was possible that the single embryo in *P. totara*, on which Buchholz based his thesis, might have been an exceptional occurrence, and if so the possibility lay, in view of the definite double mitosis leading to a tetrad in *P. andinus*, that another tendency in podocarps might be shown in advanced forms such as *Pherosphaera* and *Eupodocarpus*—a change from a primitive tetrad formation to the 2-celled unit type claimed for *Pherosphaera*. For these reasons it was necessary to use *P. nivalis* definitely to confirm or reject Buchholz's thesis as far as *Eupodocarpus* was concerned. As was shown in the second paper of this series the thesis was fully confirmed.

What then is the position in podocarps in general? As a double mitosis in the binucleate *E* cells, giving a tetrad of uninucleate cells, has been demonstrated for one species of *Stachycarpus* and two of *Eupodocarpus* it is reasonable to infer that it is a feature of the whole *Podocarpus* genus, and that any records of 4-celled uninucleate embryo groups within the genus may be taken as records of true *embryo-tetrads*. Such seem definite from Buchholz's (1941) figures for *P. urbanii* (Fig. 13), *P. macrophyllus* (Fig. 23), and *P. dacrydioides* (Fig. 8), the latter extending the occurrence to another section of the genus.

In view of the earlier claim made for *Saxe-Gothaea*, on practically no evidence and before the position had been clarified, this form has been re-examined in proper detail. It seems important to record, though the details have not yet been published, that a typical double mitosis leading to typical tetrads occur also in the *E* cells of this genus.

Similar tetrads are more than probable in *Dacrydium cupressinum* judging from the figures given by Buchholz (1933) in his account of later embryogeny and

cleavage in that species. This account was published long before he formulated his thesis or appreciated the implication of 4-celled embryonic groups. Appearances in *Dacrydium* are a little confusing, as some of the *E* cells do not go beyond the binucleate stage and a number of those that do may lag in development behind the others, but, when compared with known stages in actual tetrad formation, many of the cell groups in his Figs. 3–6 of *D. cupressinum* are unmistakeably tetrads which must have originated as the tetrads do in *Podocarpus* and *Saxe-Gothaea*.

The certain occurrence of typically developed *embryo-tetrads* in *Podocarpus* and *Saxe-Gothaea*, and their more than probable occurrence in *Dacrydium*, necessitate urgently a revision of *Pherosphaera*. Pending that revision the claim for its exceptional behaviour is not accepted. Through the courtesy of Dr. Elliot an opportunity was given for a personal examination of some of the relevant sectioned material upon which the claim for the early organization of 2-celled units was based. This is hardly the place to give a detailed criticism of the material, preparation by preparation, or to offer suggestions for future investigations. It must suffice to say, from the experience gained in the study of normal tetrad organization in *Saxe-Gothaea* and two species of *Podocarpus*, that the material was altogether too meagre in amount, and too noncommittal in nature, to allow any conclusions to be drawn. Actually from early material of *P. andinus*, used in the investigation of that species, preparations could be selected which could be interpreted as 2-celled units as easily as in the available *Pherosphaera* material. In dealing with any critical phase in conifer embryogeny there is ordinarily necessary a relatively considerable amount of material showing a close series of stages before, during, and after the critical period, before any phase can be legitimately interpreted. Such is just not yet available for *Pherosphaera*, and, viewing the meagre available material in terms of *Saxe-Gothaea* or *P. andinus*, the opinion is ventured that a full study will not substantiate the claim, though of course there are no *a priori* reasons against it. *Pherosphaera* might well have developed an exceptional course of behaviour, and the proof of it would be an observation of very great interest, but, against the rest of the podocarps, the proof would need to be cogent.

The dissected embryos (illustrated in Elliot, 1948) were not available for personal examination so no formal opinion can be expressed on them. It is not always clear from what stage of suspensor growth the figures have been taken, but most of them seem to be of rather later stages at which embryo cleavage proper is being initiated, thus of early post-tetrad stages. Here too the data are not sufficiently close or complete to convince. In the second paper of this series (Boyle and Doyle, 1954) Figs. 26 *a*, *b* show how a true tetrad could be interpreted as two 2-celled units even when only one *E* cell was present as in the illustrated example from *P. nivalis*. With many *E* cells overlapping appearances might be even more in favour of 2-celled units, and, in the absence of caps, a short run of such embryos in a dissected series might seem almost to demand Elliot's interpretation, although that interpretation would in fact be incorrect. The binucleate cells figured by him in embryo clumps in which cleavage is being initiated recall too the nondeveloping *E* cells in *P. nivalis* (Boyle and Doyle, 1954, Fig. 31) or, even more so, those in *Dacrydium cupressinum*

(Buchholz, 1933, Figs. 6, 7). On the whole, indeed, Elliot's figures for these later embryos of *Pherosphaera* seem to show a remarkable similarity to the *Dacrydium* figures given by Buchholz. It might also be mentioned that Elliot (1950) has noted how, during suspensor elongation, the embryo cells of *Pherosphaera*, whatever their origin, enter a resting stage comparable to that recorded for *P. andinus* (Looby and Doyle, 1944 b) and *P. nivalis* (Boyle and Doyle, 1954). This may be further suggestive of a similarity of *phase* at this stage between *Pherosphaera* and *Podocarpus*.

In general then, pending reinvestigation of *Pherosphaera*, it is taken here to be another general characteristic of podocarpean embryogeny that the binucleate *E* cells remain quiescent during suspensor elongation, and that then each *E* cell gives rise to an *embryo-tetrad.*

The use of the term *tetrad* has been objected to (Elliot 1950) on the grounds that the term should be kept to describe the four spores formed by meiosis from the gynospore mother cell in the ovule. The force of this objection is lessened, however, by the consideration that in a surpisingly high proportion of conifers only *three* spores are formed in the ovule, making it difficult to apply the term *tetrad* in such cases. A new term is needed which will include both a *triad* and a *tetrad* of spores. However, to meet the objection, this characteristic podocarpean feature has been here referred to as the *embryo-tetrad* stage, thus avoiding any possibility of confusion with the *spore-tetrad* in the ovule. The term suggested by Elliot, 4-celled *unit*, is to be deprecated for general use as it implies too much. It carries the implication of the acceptance of Buchholz's well known thesis as to the nature of cleavage polyembryony. Cleavage, for Buchholz, is a basal phenomenon in conifers, in which, by nature and by descent, the primary embryo cells are separate embryo initials each of which originally gave rise to a distinct embryo. This reputed primitive condition is supposed to have been retained in many living types. Those others, in which the primary embryo group of cells directly develops into a single embryo, as in *P. andinus*, *Austrotaxus*, *Cephalotaxus*, *Picea* or *Pseudolarix*, are reputed to show a derived condition, the single embryo being called a ' compound ' embryo. If this thesis is not accepted, and for reasons to be discussed elsewhere it is not here accepted, the term 4-celled *unit*, suggesting as it does that the primary embryo cells are by nature independent units, cannot be accepted either.

(*E*) *Cleavage polyembryony.*

As all members of the Podocarpaceae develop more than one archegonium simple polyembryony is common. In *P. nivalis*, for instance, as many as six young embryos, from as many archegonia, have been met with in one ovule. No analysis, however, has yet been made of the factors which determine that only one embryo per seed normally develops to maturity, and as nothing has been added to this problem in these studies no further reference need be made to it.

Cleavage polyembryony is also well developed in the Family. As it is hoped in the near future to consider critically the general problem of the nature of embryo

cleavage in the conifers as a whole, comments are here confined to little more than a factual summary of the conditions in the Podocarpaceae, giving primary attention to cleavage of the actual embryo group. The problem of suspensor proliferation, which probably occurs in most members of the Family, is here considered to be a different phenomenon not rightly included under cleavage polyembryony. It is considered to be a problem of much less importance and one which must be kept distinct from *embryo* cleavage proper. The so-called ' rosette ' cells, walled cells occurring just above the suspensors, have been sufficiently considered earlier in this paper. Such cells are not very common in podocarps, though they probably occur sporadically in all the genera, but they, too, even if they proliferate slightly, are not considered an expression of cleavage polyembryony proper. There seemed then no advantage in listing here in detail the occurrence of ' rosette " cells or proliferating suspensors within the Family, or in listing the minor variations in development shown by them.

This brief survey then is limited to the consideration of cleavage in the embryo group proper. In the second part of this series (Boyle and Doyle, 1954) the nature of the cleavage occurring in *P. nivalis* has been followed, and it has been shown to be a definitely organized process. Each *embryo-tetrad* develops into a potential embryo clump. If there is only one such tetrad there is no cleavage. If two or three, three being the normal maximum for *P. nivalis*, there will be two or three potential embryos which are separated independently by the elongation of the proximal cells of each as embryonal tubes. Since each *embryo-tetrad* is directly derived from a binucleate *E* cell each supernumerary embryo can be traced back to one of the primary embryo cells of the early proembryo. It was shown too, that, on occasion, one or more of the *E* cells may not develop, or one of the *embryo-tetrads* may lag behind the others. It is a safe assumption, wherever cleavage occurs in the Family that it is of this type, even though the early stages may not have been demonstrated. It may further be stated, though the thesis will be discussed elsewhere, that this type of polyembryony is but a form, modified in podocarps in relation to the binucleate condition of *E* cells, of a plan which is the common plan in conifers with cleavage and from which other types of cleavage described may be considered to have been derived.

But such cleavage is not universal. Those primary embryos which have a marked or at least a definite tiered arrangement of the *E* cells, that is those embryos which have been taken to be more primitive, do not normally show cleavage. In these, though there may be up to 15 *E* cells and so up to 15 *embryo-tetrads*, as in *P. andinus*, the whole group ultimately functions as one unit organization, and gives rise to one embryo. This is definitely the condition for *P. andinus* (Looby and Doyle, 1944 *b*), *P. spicatus* and *P. ferrugineus* (Buchholz, 1936),—three lower species of the *Stachycarpus* section. It is the condition in *Phyllocladus alpinus* (Buchholz, 1941) and apparently also in *Microcachrys* (Lawson, 1923), whose taxonomic position may be uncertain but whose embryo group is still definitely tiered. In *Saxe-Gothaea* also (Doyle and Looby, 1939) development ordinarily proceeds to one unit embryo, though in a small percentage of cases a slight lobing of the embryo is reported.

No proper cleavage occurs however, the result being a somewhat irregular mass of cells with a lobed tip. These unusual embryos seem to progress no further.

It is then not in these simpler forms but in the definitely advanced or somewhat advanced forms that cleavage occurs. Thus it is recorded for (*a*) the higher species of *Stachycarpus*, at least *P. amarus* (Buchholz, 1941), (*b*) the *Nageia* section (Buchholz, 1941 ; Tahara, 1941), (*c*) the *Dacrycarpus* section (Buchholz, 1941), (*d*) *Eupodocarpus* (Boyle and Doyle, 1954, *et alia*), (*e*) *Dacrydium cupressinum* (Buchholz, 1933), and (*f*) *Pherosphaera hookeriana* (Elliot, 1948, 1950). Without in any way taking up the problem of the nature of cleavage a few points arise in connection with some of these records which merit brief comment.

In the higher stachycarpean forms cleavage has thus been formally reported for one of the highest, *P. amarus*. The only stages available in this case were quite advanced, the supernumerary embryos already showing massive secondary suspensors, so the early origin has still to be followed. It is of interest however that the original suspensor system may have as many as eleven supernumerary embryos associated with it, indicating that most of the *E* cells must have at least *initiated* an embryo, though of course in these later stages only a couple of them would be advancing, the others being more or less completely retarded in development. This case of *P. amarus* is the only important one for which Buchholz gives no figures, but from the descriptive detail the record must be accepted as authentic. There is some doubt however concerning the other higher stachycarpean species such as *P. usumbarensis* and *P. gracilior*. In an earlier paper Buchholz (1936) had definitely stated that *P. usumbarensis* showed no cleavage, yet in the later paper (1941) he wishes to include both it and *P. gracilior* with *P. amarus* as forms with cleavage. Since no evidence is produced to discount the original statement that there is no cleavage in *P. usumbarensis*, and since only young stages of *P. gracilior* with quite short suspensors were available, cleavage can only be attributed to these by inference. These two species, as has been already noted, show an advance in having an embryo group of many *E* cells arranged in one tier. It is assumed, apparently, that the *E* condition in *P. amarus* is similar, as it probably is, and the inference is that if such a condition is accompanied by cleavage in this latter form then it should be accompanied by cleavage in the other two. As far then as the actual evidence goes cleavage has in fact been demonstrated only for the highest form *P. amarus*.

Cleavage has been reported in the *Nageia* section for *P. nagi* by Tahara and for *P. blumei* and *P. nankoensis* by Buchholz. In none have the details of early origin been followed. Buchholz's material was quite advanced, but Tahara's Figs. 11 and 12 of somewhat younger stages of *P. nagi* indicate that the origin of the supernumerary embryos may safely be taken as typical. Again too in this group most of the *E* cells must have initiated embryos, since the *E* number recorded for younger stages of *P. nankoensis* is 9–11, and for a later stage Buchholz records a case in one ovule of five entangled embryo systems with the remains of approximately fifty associated embryos. There seems to be nothing of the nature of the ' determinate cleavage ' described by Buchholz for *Dacrydium*, all the *E* cells seem in fact equipotential in early development. It is probably because of the definite occurrence

of cleavage in this group, in which also the *E* arrangement is one of many cells in one tier, that Buchholz *inferred* its occurrence in higher stachycarpean forms other than *P. amarus*.

Cleavage has been reported in the *Dacrycarpus* section for *P. dacrydioides* and *P. imbricatus*. These show an even more advanced *E* stage than the *Nageia* forms, *P. dacrydioides* with 3–6 and *P. imbricatus* with only 3–4 cells, in both cases in one tier. Buchholz sees in *P. dacrydioides* a form of embryogeny somewhat similar to that described by him in *Dacrydium* (1933) as ' determinate '. In this *Podocarpus* species one of the *E* cells, which may be at the side and not necessarily central, is said to be commonly larger than the others, and to take immediate precedence in the organization of an embryo. The others may not advance beyond the binucleate stage, or may only initiate very early development. This claim for early *E* cell determinancy is based however on what is clearly a very limited set of observations. The recorded case may be typical or may be only one of a range in which, in other cases, most or even all of the *E* cells may initiate relatively equal development. The position may in fact be somewhat as in *P. nivalis*, where, if say 3 *E* cells occur, all three may show equal embryonic initiation or one or two may lag in development perhaps even markedly. Examination of a much wider range of material is necessary to decide whether *E* cell determinancy in any strict sense occurs.

Much the same criticism can be applied to Buchholz's account of embryogeny in *Dacrydium cupressinum*, and to Elliot's account of *Pherosphaera hookeriana*. In *Dacrydium*, according to Buchholz, there are 5–9 *E* cells lying in two tiers with only one or two cells in the lower tier. The cells of the lower tier, especially if only one, rapidly outstrip those in the upper tier, and very early become dominant. The upper tier cells lag, sometimes remaining binucleate or at the tetrad stage, sometimes getting to an early post-tetrad stage but no further. This is the ' determinate ' cleavage of Buchholz, the future successful embryo being determined by location, all the more clearly so if only one *E* cell is present. Buchholz considers this an advanced condition, a claim which must be accepted if the condition is confirmed. But again embryogeny must be examined over a much wider range of material and in other species of *Dacrydium*. Thus Sinnott (1913) figures what he calls ' budding' in the same species (his Pl. 9, Fig. 49). This figure shows, attached to the one suspensor system, only three embryo groups, all at an advanced post-tetrad stage. Of these three the central is admittedly larger than the other two but only a very little larger, so that initially there can have been little differences between them in development. There are no indications of non-developing retarded *E* groups in an upper tier, and Sinnott's case suggests a development from a small number of *E* cells in one tier. In all podocarps with cleavage, growth rate differences between potential embryos are probably established quite early, and this figure of Sinnott's could be duplicated from *P. nivalis*. Some degree of determinancy could well be a feature of embryonic advance in *Dacrydium*, and if confirmed would be a feature of some interest. The point being made here is simply that too little is yet known of development in *Dacrydium*, or of possible variations in development in its different species, to permit any definite statement based only on the meagre data available from observations

on a few embryos from a single species. This is especially so in the light of the available records by Elliot on *Pherosphaera*, even though these in themselves are slight enough. The *E* group in *Pherosphaera*, as already noted, may show only a few cells in one tier, in which case the developmental sequence in cleavage must be practically eupodocarpean, or the *E* group may be more numerous and arranged in two tiers much as described for *Dacrydium*. Undoubtedly some of the cell groups lag in development, perhaps even markedly, but most of the groups seem to be initially practically equipotential.

It is not proposed here to pursue this question further. It has been mentioned, in a preliminary way, to call attention to the necessity of an investigation of podocarpean embryogeny on a much wider basis before the real nature of *E* cell determinancy can be clarified. There may be some *E* cell determinancy, but the importance attributed by Buchholz to ' determinate cleavage ' in relation to cleavage polyembryony in general, though the future may show it to be a useful concept, cannot be accepted as established on the available evidence.

Apart from this and other problems, difficult and perhaps insoluble, which arise in any consideration of the nature of cleavage in conifers, its broad occurrence in podocarps seems to indicate indubitably that cleavage is a derived phenomenon. If basal in conifers it is difficult to see how it could be obliterated so completely in the simpler forms and retained in the specialized and advanced types. The taxads show an interesting parallel. Again the simpler forms such as *Austrotaxus* (Saxton, 1934 ; Buchholz, 1940) show no cleavage, which is absent too in normal *Taxus* (Sterling, 1948 ; Sugihara, 1945). At most in *Taxus* an occasional embryo may split irregularly in the sense that an indefinite number of suspensors may break away from the others, and may, though not always or necessarily, carry with them an indefinite number of the embryo cells, but there is no organized cleavage. It is of interest that both genera, but especially *Austrotaxus*, show also a storied arrangement of the embryo group. In strong contrast, *Torreya*, which along with taxonomic advance shows a reduction in the number of free embryonic mitoses and in the tiering of the primary embryo cells, shows also an organized cleavage (Buchholz, 1940 ; Tahara, 1940) of a type which essentially resembles in plan that of the podocarps. There is thus no evidence from podocarps or taxads to suggest that the single unit embryo shown by the lower *Stachycarpus* species, or *Phyllocladus*, or *Austrotaxus*, or *Cephalotaxus*, is derived from a condition in which every primary embryo cell was basally a true embryo initial, originally in fact giving rise to an actual embryo. There is no evidence to suggest that those uncleaved embryos are ' compound ' embryos—all points the other way.

It is now many years since the view here taken of the nature of the embryonic group in the simpler podocarps was first expressed in general terms. " Buchholz would seem to be obsessed with the idea that every cell of the embryonic group is an actual embryo initial. Rather are they to be considered as potential embryo initials. The cells resulting from the division of the animal egg may be separated by various agencies, and each may give rise to an embryo, but there is no question here but that all the cells resulting from the egg cleavage normally form a single

embryo, and are destined so to do, in spite of the fact that they occasionally separate and show the potential embryonic tendency of each cell " (Doyle and Looby, 1939, p. 140). There is no reason on the evidence since produced to modify that general statement. One primary and obvious problem in conifers is the elucidation of the factors controlling the normal development of the simple unit embryo. Such a development as that of *P. andinus* is clearly related to the organization of a condition of marked polarity—the production of the *upper, suspensor* and *embryo* cell groups with their vastly different behaviours. Why the suspensors elongate and embryo cells do not is, for instance, a problem for which no solution can presently be offered. Examination however of the *E* group seems to show two stages in the young embryos of these forms which organize a unit embryo from many *E* cells. At the first stage the *E* cells, developed as *embryo-tetrads* though remaining in contact, are rounded and expanded, giving an impression of independence. In a second stage, and rather suddenly, they come as it were under an organizing influence which pulls them together and hammers them into the unit embryo, as if a polarity field became functional. Cleavage seems to result from some change such as a later onset of the organizing influence, or from what might be looked on, for lack of any clearer concept, as an increasing lowering of organization potential. In the absence of an early functioning of the polarity field the cells, potentially independent, become in fact independent, developing separately—some remaining dormant, some developing rapidly, some slowly—and it is in an analysis of the nature of the organizational breakdown that the solution must be sought for the slightly different behaviour of the cells in, say, *Podocarpus nagi, Dacrydium,* and *Pherosphaera.* It is in terms of such concepts that ' determinate cleavage ' for instance, if it exists, must be interpreted. The whole problem is thus one of the organization of an embryonic field controlling early development, and the elucidation of the factors which determine that control in some cases is exercised later in development, or more weakly, as a result of which equipotential cells may initiate independent activity. And if cleavage arises from such a breakdown of organization it may even be a sign of incipient degeneracy, and an indication that perhaps in six million years from now any conifer will be as rare under natural conditions as *Ginkgo* and *Metasequoia* are to-day.

Ignoring however such broad problems for the present, it is here claimed, as a statement of fact, (*a*) that basal in podocarps is an embryogeny without cleavage and (*b*) that cleavage is but another developmental tendency which has arisen in the Family, which appears in most of the advancing lines, and which is shown in a similar form even in types as widely divergent as *Eupodocarpus* and *Pherosphaera.*

General Conclusions.

Accounts had earlier been given of development in *Podocarpus andinus,* one of the lower members of the *Stachycarpus* section of *Podocarpus* (Looby and Doyle, 1944 *a, b*) and of *Saxe-Gothaea,* representative of a quite different line in the Podocarpaceae (Looby and Doyle, 1939; Doyle and Looby, 1939). In the first two papers of this series (Boyle and Doyle, 1953, 1954) and in the preceding section

of the present paper, this work was continued to include in rather full detail a study of development in *P. nivalis*, representative of *Eupodocarpus* the higher section of *Podocarpus*. In these latter accounts it seemed opportune, in view of other recent work on the podocarps, to make a comparative survey of development in the Family against this background of increasing knowledge. Such a survey strongly emphasized the known fact that the podocarps show a greater range in gametophytic organization and embryogeny than that shown by any other conifer family. The Podocarpaceae give the picture of an old Family within which, from a basal type of organization, several different evolutionary tendencies have developed, and it seemed advisable to attempt an analysis of such developments. An attempt has been made, then, in the present series to suggest the nature of the gametophytes and embryogeny in a basal podocarp type, and to offer an analysis of the various tendencies which may have affected development in the different lines in the Family. The suggestions must be considered in the light of the statement made on p. 204 of the first paper of this series (Boyle and Doyle, 1953). It is again to be emphasized that the occurrence of particular modifications of gametophytes or embryo need in no way reflect systematic affinity. Just as oogamy has developed independently in different algal classes so too any one of these tendencies may be expressed separately in different podocarp lines. As an example the general similarity in the arrangement of the primary embryo cells in *Dacrydium cupressinum* (Buchholz, 1933) and *Pherosphaera hookeriana* (Elliot, 1948, 1950) simply indicates that the same tendency in embryonic advance is shown in both, even though these two forms may in fact be the end products of two lines which have evolved independently from a very early period. Conversely, forms reasonably akin may show quite considerable differences. Thus the whole sequence in *Podocarpus andinus* differs in almost every feature from the sequence in *P. nivalis* but shows remarkable similarities with that in *Saxe-Gothaea*.

These suggestions of course can only be provisional, and are put forward merely as a first approximation to a crude picture for consideration. Even though the summary notes following are written in dogmatic form to avoid the repetition of such phrases as " it is probable ", the purely provisional nature of the suggestions must be understood. There are still too many pieces missing from the puzzle, gaps which have been sufficiently commented on in the course of the descriptions, and then again only major features have been considered.

In the course of this series of papers the developmental tendencies under review have been examined separately as they arose, and it seems necessary to give this general summary of the results in order to simplify the picture. The reputed primitive podocarp type, the extracted " common ", is first outlined, and then the developmental tendencies listed, with brief notes where necessary. The distribution and occurrence of the features concerned are set out in Table 2 for convenience of reference.

A. The Basal Podocarp Type.

Basally the gametophytes developed over two years, with a long rest period, fertilization being effected in the second growing season. This condition is still

shown by, at least, the *Stachycarpus* section, *Saxe-Gothaea*, and many species of *Dacrydium*.

In the male gametophyte the pollen grains showed one or more flotation sacs in association with the pollination of an inverted ovule secreting pollination fluid. Two prothallial cells were formed whose nuclei were permanent, were freed at germination into the cytoplasm, and then passed into the pollen tube, which, in the first year, penetrated only a short distance into the nucellus before entering the rest period. The body cell lagged markedly behind the other nuclei, overwintered in the grain, and only passed into the tube on resumption of growth. Later it gave rise to two equal male cells with relatively large nuclei. After penetration of the nucellus the tube splayed out as a disc over the top of the female gametophyte, and then, by burrowing over the archegonium, formed the supra-archegonial cavity still characteristic of all living podocarps.

The female gametophyte was much as it is to-day in *Saxe-Gothaea* or *P. andinus*. Common to all investigated species is a single gynospore mother cell. There is little trace in the Family of supernumerary mother cells, so the clean fixation of this condition must have developed early in the roots of the podocarps. A good nucellar tapetum was present. The first development of the gynospore was a very intense vacuolation even before the first nuclear division, and the resting stage was passed as a small more or less spherical vacuolate prothallus with peripheral nuclei. On growth resumption and cellular formation the change from the younger spherical to the adult rather narrow elliptical form was brought about by the peculiar meristematic activity of the upper part of the gametophyte producing the characteristic " cone ". This was one factor in determining archegonial length. The apical archegonia were few in number, up to three, long and rather pointed though perhaps not as extremely so as in the living *Saxe-Gothaea*, separate and each surrounded by a distinct jacket layer. The necks were one-tiered and flush with the surface of the gametophyte or at most sunk in a shallow pit. A ventral canal nucleus was formed if not a ventral cell. A thick two-layered membrane enclosed the gametophyte. At maturity, i.e. at fertilization, fusion took place in the upper part of the archegonium, and the egg nucleus was markedly but not grossly larger than the fusing male. All these features of the female gametophyte are still essentially shown by the lower species of the *Stachycarpus* section, by *Saxe-Gothaea*, and by at least some species of *Dacrydium*.

After fertilization at least 5 free mitoses took place of which at least 2 occurred in the position of the fusion nucleus. The resulting nuclei, at primary wall formation, became organized into a lower group of *primary embryo cells*, up to fifteen or more in number and showing a markedly storied arrangement, with above the single *open* tier. An *internal division*, affecting all the nuclei, gave a three-tiered condition—the *upper* tier *U*, the *suspensor* tier *S*, and the storied group of many *binucleate embryo cells*, *E*. The *E* cells remained quiescent during suspensor elongation, and then each produced, by a double mitosis and subsequent membrane deposition, a group of 4 uninucleate cells—the *embryo-tetrad*. The group of tetrads then organized into

one unit embryo *without cleavage*. This condition is still shown by the lower *Stachycarpus* species.

B. Gametophytic tendencies.

The following tendencies appear :—

1. A shortening of the cycle of development to one year by the dropping of a rest period, fertilization occurring in the same season as pollination. This clearly affects both gametophytes. This is shown within Podocarpus by the *Nageia*, *Dacrycarpus*, and *Eupodocarpus* sections, by *Phyllocladus*, *Pherosphaera*, and *Microcachrys*, and possibly some species of *Dacrydium*. The two contrasting groups may be referred to as two-year and one-year types.

2. A reduction of one of the male cells to a nucleus partly extruded from, but still attached to, the single functioning male cell. Two equal male cells are retained in *Saxe-Gothaea*, and possibly in *Microcachrys*, but otherwise this tendency is expressed throughout the Family.

3. A secondary increase in the number of the prothallial cells by multiplication of one or both of the basal pair, with a subsequent increase in the number of free nuclei in the tube. This affects all genera and sections, except perhaps *Microcachrys* which is reported (Lawson, 1923) to have only two and *Pherosphaera* which has none. Apart from the rest period in the one case and the uninterrupted growth of the pollen tube in the other the behaviour of the nuclei, especially the body cell, is similar in both the two-year and one-year types, and neither show any general modification from that described for the basal podocarp.

4. In relation to special pollination arrangements modifications are to be seen in the flotation sacs of the pollen in certain genera, notably the wingless condition of the grains in *Saxe-Gothaea*, but these have been sufficiently dealt with elsewhere (Doyle, 1945).

5. The development of shorter archegonia with blunter ends replacing the long narrow primitive type still shown in the *Stachycarpus* section, *Saxe-Gothaea* and at least some species of *Dacrydium*. This tendency is shown particularly by the *Eupodocarpus* section, *Pherosphaera* and *Microcachrys*, an intermediate position being taken by *Phyllocladus*, the higher *Stachycarpus* species, and possibly some higher species of *Dacrydium*. The full range of this tendency and its expression in detail has still to be worked out.

6. An increase in archegonial number. This is most strikingly shown in *Eupodocarpus* and *Nageia* and also, though less markedly, in *Microcachrys* and *Pherosphaera*. The number, 1–3, is still low elsewhere in the Family. Archegonial form is still uncertain in *Nageia* but otherwise where archegonia are numerous they tend to be smaller.

7. Deep early embedding of the archegonia.

8. Occurrence of a large egg nucleus producing a gross inequality between the fusing nuclei at fertilization.

These latter two (7, 8) seem to be special developments known to occur in the lower *Stachycarpus* species and possibly shown, but certainly less markedly, in *Dacrycarpus*.

TABLE 2.

Distribution of Primitive and Advanced Features in Podocarps

+, character shown ; ?, unknown or very uncertain ;
+ ?, possible but uncertain and due for revision.
Primitive characters indicated by P.

		Stachycarpus (lower)	*Stachycarpus (higher)*	*Nageia*	*Dacrycarpus*	*Eupodocarpus*	*Saxe-Gothaea*	*Dacrydium (colensoi type)*	*Dacrydium (cupressinum type)*	*Pherosphaera*	*Phyllocladus*	*Microcachrys*
2-year type ...	P.	+	+				+	+	+			
1-year type ...	...			+	+	+				+	+	+
Male cells equal ...	P.						+					+?
Prothallial cells many	...	+	+	+	+	+	+	+	+	[0]	+	[2]
Archegonia few (up to 3)	P.	+	+				+	+	+		+	
Archegonia up to 6	...									+		+
Archegonia many	...			+	+	+						
Archegonia long, narrow	P.	+		?			+	+				
Archegonia intermediate	...			+	? +?			+		+		
Archegonia shorter, blunter					? +?	+				+		+
Archegonia separate	P.	+	+	+	+	+	+	+	+	+	+	
Archegonia flush ...	P.		?	?	+?	+	+	?	+	+?	+	+
Archegonia embedded	...	+	?	?	?					+		
Fusing nuclei not grossly unequal, or subequal	P.		?	+	+?	+	+	+	+	+	+	+
Fusing nuclei grossly unequal		+	?		+?							
5 free mitoses, 2 above	P.	+	+	+				?				
4 free mitoses, 2 above	...						+	?				
4 free mitoses, 1 above	...				+	+		+?	+?	+	+?	+?
E cells storied ...	P.	+					+				+	
E cells in two tiers	...							?	+	+		+
E cells in one tier	...		+	+	+	+						
E cells many, up to 15	P.	+	+	+			+				+	
E cells about 4–7	...				+			+	+	+		+?
E cells few, up to 3	...					+						
No cleavage ...	P.	+					+	?			+	+
Cleavage occurring	...		+	+	+	+		?	+	+		

9. Extreme thinning of the gametophyte membrane. This seems to occur definitely in *Pherosphaera*, but a firm membrane seems to be retained as a general family feature. A slight thinning may have supervened in the *Nageia* section. But this character needs proper revision.

There may perhaps be other tendencies appearing when sufficient forms have been adequately investigated.

Thus all properly known forms with definitely short archegonia are one-year types. In these the gametophyte seems to be elliptical from the start, and the shortness of the archegonia seems related to the relatively slight activity of the upper meristematic zone in such species. Possibly a change from a young spherical gametophyte to a young elliptical gametophyte may have to be included as another general tendency connected with a shortening of the life cycle, but data are still wanting especially for example with reference to the higher *Stachycarpus* species, such as *P. usumbarensis*, which though two-year types, are reported to have shorter archegonia with blunter ends.

Again in *Eupodocarpus*, at least, the intense vacuolation of the gynospore on first germination is replaced by a degree of vacuolation which is about normal. This may also be a minor widespread change related to the shortening of the life cycle but present data are utterly inadequate.

C. Tendencies in embryogeny.

In *P. andinus* and *P. nivalis* there has been demonstrated the extrusion of a variable number of embryonic nuclei at the free nuclear phase. Whether such an extrusion was characteristic of the primitive podocarp is of course quite uncertain, but it is probably widespread among living podocarps, and accounts largely for the great variation in *S* and *E* numbers recorded for any type. A species will show, not a definite determined *S* and *E* number, but a range which may be narrow or wide. The details however concerning such extrusion remain still practically uninvestigated in most types. Ignoring the occurrence of extrusion the following tendencies in embryogeny can be seen.

10. The dropping out of 1 of the free mitoses, giving 4 free mitoses in place of 5. The more primitive condition of 5 free mitoses of which 2 occur above is still shown by the species of *Stachycarpus* and *Nageia*. In all other forms, except *Saxe-Gothaea*, this seems to be replaced by 4 free mitoses of which only 1 occurs above. In *Saxe-Gothaea* there are also 4 free mitoses but 2 of these occur above.

11. The strongly storied arrangement of the *E* cells passes into a condition showing the *E* cells in one tier. The primitive condition is still shown by the lower *Stachycarpus* species, by *Saxe-Gothaea* and by *Phyllocladus*. The arrangement of the *E* cells in one tier appears in the higher *Stachycarpus* species, *Nageia*, *Dacrycarpus* and *Eupodocarpus*. It is thus best expressed within *Podocarpus*. An intermediate stage of two unequal tiers is shown in *Dacrydium* and *Pherosphaera*. *Microcachrys* probably shows two equal tiers.

12. Reduction in the number of *E* cells. Many *E* cells are still shown by the species of *Stachycarpus* and of *Nageia*, by *Saxe-Gothaea*, by *Phyllocladus*, and probably by *Microcachrys*. An intermediate number is shown in *Dacrycarpus*, *Dacrydium*, and *Pherosphaera*, the extreme reduction developing in *Eupodocarpus*. In most cases this tendency coincides with tendency 11 but in the higher *Stachycarpus* species and *Nageia* the *E* cells though in one tier are still numerous.

13. The development of organized cleavage. The primitive absence of cleavage is still a character of the lower *Stachycarpus* species, of *Saxe-Gothaea*, *Phyllocladus*, and *Microcachrys*. Cleavage, of the type described in this series of papers, is shown by *Dacrydium*, *Pherosphaera*, and the rest of *Podocarpus*, although its occurrence in the higher *Stachycarpus* forms other than *P. amarus* is doubtful. A minor tendency may be the development of 'determinate' cleavage, especially in *Dacrydium*, but the question of 'determinate' cleavage needs complete revision.

Finally, although *Saxe-Gothaea*, among coned podocarps, is systematically advanced in some directions, notably in the extreme reduction of the axillary part of the cone unit and in the special pollination arrangements, its gametophytes and embryogeny, except in one particular, are those of the reputed primitive type. Instead of 2 free mitoses above and 3 below it has only 2 below thus giving a smaller number of embryo cells. Had *Saxe-Gothaea*, like *P. andinus*, the full 5 mitoses it would be a replica of the primitive type except perhaps for the extreme tapering of the archegonia. From such a type the various modifications in the Family can readily be interpreted in the light of the developmental tendencies outlined. It is difficult to relate all the modifications satisfactorily to any other basal concept.

Table 2 is based, for the time being, on the idea that the present genera are the survivors of separate lines distinct in evolution from relatively early times. This tabular form seemed the most convenient way to record the occurrence of the various features here discussed. The more primitive characters retained and the advanced tendencies shown in each line can be read from it, and the expression of the various tendencies within *Podocarpus* can also be readily determined. Even if later it is shown that much closer relationships exist between some of these lines the Table can still stand as a convenient progress record.

SUMMARY

In the first part of this paper the post-fertilization embryogeny including cleavage embryogeny, which was shown in the second paper to be typical of *Eupodocarpus*, is further compared with the available data for embryogeny in the rest of the Podocarpaceae, to determine the nature of the basal embryogeny and of the derivative tendencies in embryonic advance in the Family. The greatly varying embryogenies in the Family show an underlying common plan, and are all readily derived from a basal type without cleavage.

In the second part the series of three papers are summarized, and there is put forward a provisional picture of evolutionary development within the Family of gametophytes and embryos. An outline of the probable nature of these in the primitive

podocarp is given. Analysis of the data seems to show that the great range of variation in developmental features shown in the Family can be accounted for by the operation of about a dozen evolutionary developmental tendencies, some effective in one line some in another. The distribution and occurrence of the primitive features and advanced tendencies are summarized in a Table.

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